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(71) Applicant (for all designated States except US): THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE SECRETARY, DEPARTMENT OF HEALTH & HUMAN SERVICES, THE NATIONAL INSTITUTES OF HEALTH [US/US]; Office of Technology Transfer, Suite #325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): KALLIONIEMI,

Olli, P. [FI/US]; 1083 Grand Oak Way, Rockville, MD 20852 (US). KONONEN, Juba [FI/US]; 5801 Nicholson Lane, Apartment #1021, Bethesda, MD 20852 (US). BUDENDORF, Lukas [CH/US]; DROSSELSTRASSE 31, CH-4106 Therwil (US). DOUGHERTY, Edward, R. [US/US]; 3101 Westchester Avenue, College Station, TX 77845 (US). GRIGORYAN, Artyom, M. [AM/US]; 11246 Cedar Park, San Antonio, TX 78249-4263 (US).

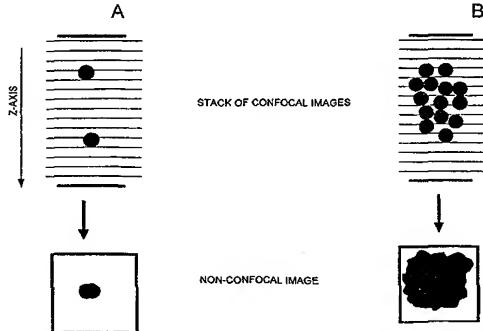
(74) Agent: NOONAN, William, D.; Klarquist, Sparkman, Campbell, Leigh & Whinston, LLP, One World Trade Center, Suite 1600, 121 SW Salmon Street, Portland, OR 97204 (US).

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(54) Title: SIGNAL COUNTING FOR IN SITU HYBRIDIZATION



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(57) Abstract: Fluorescently tagged nucleic acid probe signals are counted in biological specimens by determining a ratio of signals from a test probe to signals of a reference probe. Probe signals need not be counted with reference to cells, nuclei, or nuclear contours. Gene amplification or deletion can thus be detected by analyzing the ratio. Successive image slices are obtained by confocal microscopy, and the images are digitized. The digital images are transformed and analyzed to combine contiguous fluorescent signal segments in successive optical sections to identify discrete probe signals, or spots. Spots overlapping in the axial and transverse dimensions of a three-dimensional representation of the biological specimens can be distinguished. A graphical user interface presents various features for consideration by a user, who can provide guidance to a computer system counting the spots. Various features directed to identifying spot clusters and autofluorescent material can increase accuracy of spot counting.



patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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## INTERNATIONAL SEARCH REPORT

Internal Application No  
PCT/US 00/25465

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/68 G01N15/14

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RODENACKER ET AL.: "Groping for quantitative digital 3-D image analysis: an approach to quantitative fluorescence <i>in situ</i> hybridization in thick tissue sections of prostate carcinoma" ANALYTICAL CELLULAR PATHOLOGY, vol. 15, no. 1, 1997, pages 19-29, XP001024113	1, 6-23, 27-40, 48-63
Y	the whole document	1-26, 41-47
X	WO 99 44063 A (THE UNITED STATES OF AMERICA) 2 September 1999 (1999-09-02) page 13, line 16 -page 24, line 20; examples 1,3	1-5
Y	---	1-14, 24-26, 41-47
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 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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- \*Y\* document of particular relevance; the claimed invention cannot be considered inventive as inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
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Date of the actual completion of the international search

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.  
Fax: (+31-70) 340-3016

Authorized officer

Botz, J

## IN. NATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AUBELE ET AL.: "Comparative FISH analysis of numerical chromosome 7 abnormalities in 5- $\mu$ m and 15- $\mu$ m paraffin-embedded tissue sections from prostatic carcinoma" HISTOCHEMISTRY AND CELL BIOLOGY, vol. 107, no. 2, 1997, pages 121-126, XP001024090 the whole document -----	1,6-23, 27-40, 54-63
Y	US 5 817 462 A (GARINI ET AL.) 6 October 1998 (1998-10-06) column 26, line 45 - line 58 -----	1-14, 24-26, 41-47
Y		1-23

## INATIONAL SEARCH REPORT

Information on patent family members

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